REMARKS

I. Regarding Priority

In section 7 of the office action (p. 3), the Examiner states that claims 3-6, 8 and 10-12 are not granted priority benefit to 09/695,762 and 60/161,035 based on a contention that these claims recite subject matter not adequately disclosed under 35 U.S.C. § 112, ¶ 1 in 09/695,762. Applicants respectfully traverse this contention as follows.

Claims 4 and 10 add the further limitation "wherein said sample further comprises a chaotropic agent in an amount which is insufficient to denature said native protein at ambient pressure." "Chaotropic agent" is term of art and as expressly indicated in 09/695,762, includes agents "such as guanidine chloride or urea, and reducing agents (Cleland, 1993)." See 09/695,762, p. 2, ll. 3-4. Further chaotropic agents are described at page 3, lines 14-16 of 09/695,762. In the "Summary of the Invention" at page 2, lines 20-28, the 09/695,762 application teaches:

> It has been discovered that by application of hydrostatic pressure, protein aggregation can be inhibited or reversed. It is effective both in preventing aggregation during refolding and in reversing aggregation which has already taken place. After pressure is released, dissociated aggregates refold to form biologically active protein with native characteristics. ...
>
> An additional benefit of the present invention is that the **use of the present invention substantially** or even entirely obviates the need for urea or other denaturants.

(Emphasis added). In further summary of the invention, the 09/695,762 at page 4, lines 1-16, states in relevant part that:

> The present invention also provides a method to inhibit or reverse protein aggregation by subjecting a sample to high hydrostatic pressure, where the sample contains a protein aggregate, so as to substantially unfold the protein of the protein aggregate, and returning the sample to ambient pressure so as to allow the unfolded protein to refold, thereby recovering native protein from the protein aggregate. ....

> The invention also provides **embodiment of such methods where the sample is substantially free of a denaturing agent** selected from the group consisting of guanidine hydrochloride, guanidine thiocyanate, sodium dodecyl sulfate (SDS), and Urea. Similarly the present invention provides embodiments of such methods where the sample is substantially free of sodium dodecyl sulfate (SDS).
>
> .... Similarly, the invention provides embodiments of such methods **where the denatured protein is unfolded in the presence of a reducing agent.**

(Emphasis added). As asserted in the attached Declaration Under 37 C.F.R. § 1.132 by Dr. Anne Robinson, one skilled in the art would necessarily understand the above passages of the 09/695,762 application as indicating that some methods of the invention involve use of protein aggregates in a sample further containing "a chaotropic agent in an amount which is insufficient to denature said native protein at ambient pressure," as that phrase is used in claims 4 and 10 of the above referenced application. See paragraph 4.c. Dr. Robinson states that "by definition a denatured protein does not have the same conformation as the native protein" and that "[i]t necessarily follows that in those embodiments of the invention which use as a starting material a sample comprising protein aggregates and a chaotropic agent, that it would not be possible to recover native protein at ambient pressure following the hydrostatic pressurization step if a chaotropic agent were present in an amount sufficient to denature the protein at ambient pressure." See paragraph 4.c.

Claims 3, 5, 8 and 11 add the further limitation "wherein said elevated hydrostatic pressure is insufficient to fully denature said protein" or "said protein folding intermediates." The "Summary of the Invention" of 09/695,762 at page 4, lines 1-7, states that:

> The present invention also provides a method to inhibit or reverse protein aggregation by subjecting a sample to high hydrostatic pressure, where the sample contains a protein aggregate, so as to substantially unfold the protein of the protein aggregate, and returning the sample to ambient pressure so as to allow the unfolded protein to refold, thereby recovering native protein from the protein aggregate. **In accordance with certain embodiment of such methods, the high**

> **hydrostatic pressure is from preferably about 1 to about 3.5 kbar or about 2.5 kbar.**

(Emphasis added). In the paragraph spanning pages 6-7 of 09/695,762 states in relevant part that:

> It has been discovered that by application of hydrostatic pressure, protein aggregation can be inhibited or reversed. .... **After pressure is released, dissociated aggregates refold to form biologically active protein with native characteristics. .... The partially unfolded intermediate**s appear to preferably refold into the conformation of the native protein, rather than merely re-aggregating upon release of pressure.

(Emphasis added). As asserted in the attached Declaration Under 37 C.F.R. § 1.132 by Dr. Anne Robinson (see paragraph 5.c.), and indicated at page 6, line 18 – page 7, line 3 of 09/695,762, it was well known in art that application of hydrostatic pressure to proteins can be carried out without denaturing the tertiary and/or secondary structure of proteins. In particular, Dr. Robinson points out that at page 7, lines 1-3 of 09/695,762 (citing references), "secondary and tertiary structures of proteins typically do not denature until pressures above 5 kbar." See paragraph 5.c. Dr. Robinson further points out, in paragraph 5.d. of her declaration, that at page 9, lines 3-12 of 09/695,762, it is stated that:

> Reversal of protein aggregation by the present invention is believed to be somewhat analogous to pressure dissociation of oligomeric proteins. **The chains that are dissociated by pressure are competent for rapid productive folding, perhaps because the secondary and tertiary structure is preserved.** The pressure-sensitive interfaces of aggregates are likely to be well-packed and solvent-excluded, suggesting that aggregation involves specific protein-protein interactions.
>
> In accordance with the present invention, a sample containing a protein of interest is subjected to high hydrostatic pressure. Preferably, the hydrostatic pressure is between about 0.5 kbar and 10 kbar, preferably about 1 kbar to about 3.5 kbar, most preferably about 2 to 3 kbar.

(Emphasis added.) According to Dr. Robinson, based on the knowledge in the art as to the level of pressure required to denature the secondary and tertiary structures of proteins (i.e., above 5

kbar), one skilled in skilled in the art would immediately appreciate that the range of hydrostatic pressures applied in some preferred embodiments (i.e., 1-3.5 kbar) would be "insufficient to fully denature said protein" or "said protein folding intermediates." See paragraph 5.e. Dr. Robinson further points out that the 09/695,762 application explicitly indicates that the inventors believed that a phenomena underlying rapid productive refolding is that secondary and tertiary structure of a protein is preserved even during application of elevated hydrostatic pressure. See paragraph 5.f. Finally, Dr. Robinson concludes that "[b]ecause a 'fully denatured' protein only retains primary structure, one skilled in the art would have necessarily understood that the inventors were describing a method wherein protein aggregates were exposed to hydrostatic pressures in range of pressure which is insufficient to fully denature the protein (i.e., a pressure at which tertiary and/or secondary structure is retained)."

Claims 6 and 12 add the further limitation "said protein aggregates are inclusion bodies." It is noted that claim 9, which also recites this limitation, was not asserted to lack priority support. In any event, explicit support for this limitation in the 09/695,762 application can be found at page 3, lines 5-12; page 4, lines 26-28; page 7, lines 13-17 (defining the term "inclusion body"); page 10, lines 5-7; page 11, lines 14-21.

II. Regarding Objections to the Specification

In section 10 of the office action (pp. 4-5), the Examiner objects to the specification under 37 C.F.R. § 1.75(d)(1) as failing to provide proper antecedent basis for the claimed subject matter of claims 3-6, 8, and 10-12. Applicants respectfully traverse this objection. As noted in the above discussion with respect to the priority entitlement, the limitations in claims 3-6, 8, and 10-12 find express or inherent support in the 09/695,762 application. Since the specification of

the present application is identical to the 09/695,762, it follows that the limitations in claims 3-6, 8, and 10-12 have proper antecedent basis in the current application.

III. Regarding Objections to the Claims

In section 11 of the office action (p. 5), the Examiner objects to term "hydrostic" as recited in claims 3, 5, 8 and 11, and further notes that it appears to be a misspelling of "hydrostatic." Applicants thank the Examiner for the careful review of the claims and believe the current amendments to the claims obviate this rejection.

IV. Rejection of Claims 1-6 As Lacking Adequate Written Description

In section 13 of the office action (pp. 5-11), the Examiner rejected claims 1-6 under 35 U.S.C. § 112, ¶ 1 as lacking adequate written description. In particular, the Examiner contends that the claimed methods use

> a broad genus of compositions, i.e. protein aggregates, which represents enormous scope because the claims do not place any limitations on the number of atoms, i.e. binding moieties, types of atoms or the way in which said atoms can be connected together to form such a compound and/or composition (protein structures).

Office Action at p. 7, ll. 1-5. The Examiner further asserts that because there is no known correlation between the amino acid sequence of a protein and its three-dimensional structure, the claims encompass "virtually every known class and subclass of compounds, i.e. the protein structures of the folding and aggregate pathway for any protein." Office Action at p. 7, ll. 7-12. Still further, the Examiner contends that even though

> the instant claimed protein aggregates is [sic] further define [sic] by a functional limitations [sic], i.e. '*a portion of protein dissociates from said protein aggregates*' at high pressure

this does not provide an adequate written description since

> Applicants provide no chemical structure *for* the claimed genus of composition, i.e. protein aggregates, and only distinguish the claimed genus from others, except by function.

Office Action at p. 7, l. 13 – p. 8, l. 5. In this regard, the Examiner further contends that

> methods were not sufficiently routine or predictable at the time of filing, to permit one of skill in the art to devise strategies for the use of any protein aggregates such that '*a portion of protein dissociates from said protein aggregates*' at high pressure.

Office Action at p. 8, ll. 6-9. The Examiner further cites Panick *et al.* as teaching that the protein structure produced by pressure depends on the type of protein used as the starting material, and that the nature of the transition state(s) in denaturation of a protein may vary depending on the method employed to perturb the native structure. Office Action at p. 8, ll. 10-22.

At pages 9-11 of the Office Action, the Examiner further contends that exemplification of tailspike protein aggregates using the claimed methods, which the Examiner characterizes as a "a well-known protein in which the intermediate forms of the folding and aggregates pathway of the protein are known" is insufficiently representative of the claimed method to "to demonstrate that *applicant had possession of the full scope of the claimed invention.*" In particular, the Examiner contends that protein aggregates of tailspike folding intermediates would not convince a skilled artisan that protein aggregates of other proteins could be used in the method. See Office Action at p. 10, ll. 11-17. Moreover, the Examiner contends that "the disclosure fails to describe the common attributes or characteristics that identify members of the genus, and because the genus is highly variable, the instant specification single example is insufficient to describe the enormous genus."

For the reasons that follow, Applicants respectfully traverse the rejection of claims 1-6 as lacking adequate written description. The Examiner's primary contention is that the genus of protein aggregates which are susceptible to dissociation upon application of hydrostatic pressure is not adequately described in the specification in terms of a description of a structural feature common to all such protein aggregates. Applicants believe that the Examiner has overlooked the

knowledge in the art, as detailed below, in assessing the adequacy of the written description for the claims in the current application.

Claim 1 and claims depending therefrom are directed to a method for recovering native protein from a sample comprising protein aggregates. In the claimed methods, the sample is subjected to elevated hydrostatic pressure which mediates release of protein from the aggregate. The released protein is recovered as native protein after return of the sample to ambient pressure. The two principle phenomena underlying recovery of native protein using the claimed method are: (1) that hydrostatic pressure can be used to dissociate aggregates; and (2) that dissociated protein is capable of refolding to native protein. The Examiner contends that exemplification of recovery of native tailspike protein from protein aggregates using the claimed method would not have convinced those of skill in the art that applicants were in possession of a method that could be applied to other protein aggregates. However, in the attached Declaration Under 37 C.F.R. § 1.132 by Dr. Anne Robinson (see paragraph 7), Dr. Robinson believes that the Examiner has overlooked the knowledge in the art as of the earliest date for which priority of the current application is claimed. In particular, Dr. Robinson states that

> it was recognized in the art that: (1) protein aggregates comprise proteins having partially denatured conformations (i.e., proteins having at least secondary structure); (2) the predominant force mediating protein aggregation was intermolecular hydrophobic interactions; (3) hydrostatic pressure could be used to disrupt intermolecular interactions, and in particular, intermolecular hydrophobic interactions; and (4) proteins have an inherent tendency to refold properly if forces driving aggregation of folding intermediates are minimized or entirely disrupted.

See paragraph 7.

Dr. Robinson cites numerous references indicating that as of the earliest filing date sought for the current application that it was recognized in the art that protein aggregates in general possessed common attributes. Specifically, Dr. Robinson provides ample evidence that

the art recognized that protein aggregates are comprised of incompletely folded protein predominantly driven to associate by hydrophobic intermolecular associations. See ¶¶ 8 and 9. In view of the recognition in the art of these common attributes of protein aggregates, Dr. Robinson states that a skilled artisan would expect that elevated hydrostatic pressure could be used to dissociate protein from aggregates of other proteins, like the dissociation of protein from tailspike protein aggregates as exemplified in the current application. See ¶ 12.

Dr. Robinson also cites numerous references in indicating that as of the earliest filing date sought for the current application, it was recognized in the art that minimizing intermolecular interactions that lead to aggregation of partially folded intermediates can be an important factor in optimizing successful refolding and that denatured protein chains are capable of refolding spontaneously to a correctly folded conformation. See ¶ 11. In view of this knowledge, Dr. Robinson avers that:

> a skilled artisan would also be convinced that native protein could be recovered from protein which dissociates from protein aggregates subjected to hydrostatic pressure, similar to the native tailspike recovered following application of hydrostatic pressure to tailspike protein aggregates, since the phenomena underlying the ability of a dissociated protein to assume a native conformation by the hydrostatic pressure methods of the present invention is no different than the phenomena relied upon by every other method of recovering native protein from protein aggregates (i.e., an inherent ability of incompletely folded protein to spontaneously refold to a native conformation).

See ¶ 13.

In view of the above argument, Dr. Robinson's declaration submitted under 37 C.F.R. § 1.132, and the evidence submitted therewith, Applicants respectfully submit that the specification in view of the knowledge in the art adequately describes the methods of claims 1-6 and request that the Examiner withdraw the rejection of these claims as lacking adequate written description.

V. Rejection of Claims 1-6 As Lacking Enablement

In section 14 of the office action (pp. 12-15), the Examiner rejected claims 1-6 under 35 U.S.C. § 112, ¶ 1 as lacking enablement commensurate in scope with the subject matter claimed. In particular, the Examiner undertakes a *Wands* factor analysis, concluding that undue experimentation would be required to practice using any type of protein aggregates. Applicants respectfully traverse this rejection. In particular, Applicants disagree with the Examiner's assessment of the scope of the claims.

At page 12, ¶ 1 of the Office Action, the Examiner asserts that exemplification of the method using tailspike protein aggregates "does not reasonably provide enablement for the method of high-pressure dissociation of protein aggregates intermediate forms using *any type of protein aggregates*." (Emphasis added). In the paragraph spanning pages 12-13, the Examiner contends that the claims encompass an *infinite* genus of protein aggregates. At page 13, ¶ 2, the Examiner contends that the unpredictability in the art would not "permit one of skill in the art to devise strategies for the use of *any protein aggregates*." (Emphasis added). Finally, at page 15, ¶ 2, the Examiner states:

> Therefore based on the evidences as a whole regarding each of the above factors ..., the specification, at the time the application was filed, does not satisfy the enablement requirement for the instant claimed method of high-pressure dissociation of protein aggregates intermediate forms using *any type of protein aggregates*.

(Emphasis added).

The above statements by the Examiner indicate that the Examiner views the claims in the current application as reading on use of every possible protein aggregate. However, the claims explicitly limit the scope of protein aggregates by functional language to protein aggregates for which (1) protein dissociates from the aggregate upon subjection to elevated hydrostatic

pressure; and (2) wherein the dissociated protein assumes a native conformation at ambient temperature. Thus, the enablement inquiry, as was applied by the Examiner, is not whether the specification and knowledge in the art enable a skilled artisan to use elevated hydrostatic pressure in a method for producing native protein from *any* and *every* type of protein aggregate. Rather, the proper enablement inquiry is whether one skilled in the art could determine which protein aggregates would be suitable for use in the presently claimed invention.[1] As discussed below, Applicants submit that only routine experimentation would be needed for one skilled in the art to make this determination as to protein aggregates of any particular protein.

In paragraph 14 of the attached declaration by Dr. Robinson submitted under 37 C.F.R. § 1.132, Dr. Robinson asserts that

> In view of the teachings found in the application and the knowledge in the art as of October 25, 1999, it is my opinion that only routine experimentation would be required to determine whether protein aggregates of any particular protein are amenable for use in the currently claimed methods.

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[1] For a factually similar scenario, see *Ex parte Mark*, 12 USPQ2d 1904, 1906-7 (B.P.A.I. 1989). The application in *Ex parte Mark* contained claims directed to a method for producing a biologically active protein having a deletion or substitution mutation in at least one cysteine residue, and claims directed to the product of the method. The Examiner rejected the claims as non-enabled, asserting that it would take undue experimentation to determine which mutants of every protein retained biological activity given the complete unpredictability associated with making such a mutation. The Board held the claims to be enabled, stating that the "fact that a given protein may not be amenable for use in the present invention in that the cysteine residues are needed for the biological activity of the protein does not militate against a conclusion of enablement. One skilled in the art is clearly enabled to perform such work as needed to determine whether the cysteine residues of a given protein are needed for retention of biological activity."

As support for this conclusion, Dr. Robinson points out, that in contrast to the Examiner's assessment at page 14 of the Office Action that the application provides no other guidance than the single exemplification of the method on tailspike protein aggregates, additional guidance in the form of general teachings can be found in the current application at:

- p. 3, ll. 22-29, describing a general method for determining the optimal hydrostatic pressure to recover a native protein;
- p. 10, ll. 20-29, providing guidance on use of assays for monitoring native and non-native conformations, with specific exemplification of the use of HPLC in Example 1 of the application (pp. 14-17);
- p. 11, ll. 1-5, providing guidance on the use of reducing agents if the protein of interest contains post-translational modifications;
- p. 11, ll. 6-13, providing guidance on the use of chaperones or isomerases to assist refolding of a protein of interest; and
- p. 12, ll. 15-27, providing guidance on the temperature at which hydrostatic pressure should be applied.

Dr. Robinson also points out the extensive knowledge that existed in the art regarding techniques for monitoring native and non-native conformations of proteins, citing as evidence numerous references. See ¶ 16 of the declaration. Based on the guidance provided in the specification and the knowledge in the art in October, 1999, Dr. Robinson asserts that one skilled in the art: (1) would have been able to identify conditions under which hydrostatic pressure could be applied to cause dissociation of protein from protein aggregates comprising proteins other than tailspike using only a limited amount of experimentation (see ¶ 17 of the declaration); and (2) would have been able to identify whether native protein was recovered following return of a sample to

ambient pressure using conventional techniques (see ¶ 18 of the declaration). Dr. Robinson further asserts that a skilled artisan in October, 1999, would have considered both the determination of the hydrostatic pressure conditions under which protein dissociates from protein aggregates as well as the determination of the presence of native protein following return to ambient pressure as routine experimentation in view of the extensive experimentation carried out by the industry using other methods for recovering native protein from protein aggregates. See ¶ 19 of the declaration.

In view of the above remarks, Dr. Robinson's declaration submitted under 37 C.F.R. § 1.132, and the evidence submitted therewith, Applicants respectfully submit that the specification in view of the knowledge in the art enables the full scope of the methods of claims 1-6 and request that the Examiner withdraw the rejection of these claims as non-enabled.

VI. Rejection of Claims 7-12 As Lacking Adequate Written Description

In section 15 of the office action (pp. 15-22), the Examiner rejected claims 7-12 under 35 U.S.C. § 112, ¶ 1 as lacking adequate written description. In particular, the Examiner contends that the claimed methods use

> a broad genus of compositions, i.e. protein aggregates comprising '*protein folding intermediates of a native protein*', which represents enormous scope because the claims do not place any limitations on the number of atoms, i.e. binding moieties, types of atoms or the way in which said atoms can be connected together to form such a compound and/or composition (protein structures).

Office Action at p. 17, ll. 6-10. The remainder of the rejection by the Examiner mirrors the basis for rejecting claims 1-6 as lacking written description support. Applicants traverse this rejection.

In view of the argument, Dr. Robinson's declaration submitted under 37 C.F.R. § 1.132 (and in particular, ¶ 9.b. (averring that "protein aggregates (including inclusion bodies) generally comprise non-covalently associated protein folding intermediates")), and the evidence submitted

therewith, in Applicants' traversal of the rejection of claims 1-6 as lacking written description support, Applicants respectfully submit that the specification in view of the knowledge in the art adequately describes the methods of claims 7-12 and request that the Examiner withdraw the rejection of these claims as lacking adequate written description.

VII. Rejection of Claims 7-12 As Lacking Enablement

In section 16 of the office action (pp. 22-26), the Examiner rejected claims 7-12 under 35 U.S.C. § 112, ¶ 1 lacking enablement commensurate in scope with the subject matter claimed. In particular, the Examiner undertakes a *Wands* factor analysis identical to the analysis taken with respect to claims 1-6. Applicants traverse this rejection.

In view of the argument, Dr. Robinson's declaration submitted under 37 C.F.R. § 1.132 (and in particular, ¶ 9.b. (averring that "protein aggregates (including inclusion bodies) generally comprise non-covalently associated protein folding intermediates"), and the evidence submitted therewith, in Applicants' traversal of the rejection of claims 1-6 as lacking enablement, Applicants respectfully submit that the specification in view of the knowledge in the art adequately describes the methods of claims 7-12 and request that the Examiner withdraw the rejection of these claims as lacking adequate written description.

VIII. Rejection of Claims 3-5, 8, 10 and 11 As Indefinite

In section 18 of the office action (pp. 26-27), the Examiner rejects claims 3-5, 8, 10 and 11 under 35 U.S.C. § 112, ¶ 2 as being indefinite. In particular, the Examiner asserts that a lack of clarity exists in the metes and bounds of the phrase "elevated hydrostatic pressure" as used in claims 3, 5, 8 and 11 in reciting the limitation "wherein said elevated hydrostatic pressure is insufficient to fully denature said protein." See Section 18.a. (p. 26) of the Office Action. The Examiner further asserts that a lack of clarity exists in the metes and bounds of the phrase

"amount" as used in claims 4 and 10 in reciting the limitation of "a chaotropic agent in an amount which is insufficient to denature said native protein at ambient pressure." Applicants traverse these rejections.

Regarding the "elevated hydrostatic pressure" limitation, in view of the discussion of this limitation in Section I of the current response (Regarding Priority), Applicants submit that the term "elevated hydrostatic pressure" would necessarily be understood as a pressure between ambient and a pressure which causes complete loss of secondary structure. Techniques for monitoring the presence of secondary structure of proteins subjected to hydrostatic pressure were known as of October, 1999 and could be used to determine the upper limit defining the phrase "elevated hydrostatic pressure." See **Exhibits 30** and **36-37**, filed with the current response.

Regarding the "chaotropic agent" limitation, in view of the discussion of this limitation in Section I of the current response (Regarding Priority), Applicants submit that the term "amount" would necessarily be understood by a skilled artisan as referring to a concentration of chaotropic agent which is insufficient to cause an alteration in structural conformation of the native protein of interest at ambient pressure. As noted in paragraph 16 of the Declaration Under 37 C.F.R. § 1.132 by Dr. Anne Robinson, and evidence submitted therewith, extensive knowledge existed in the art regarding techniques for monitoring native and non-native conformations of proteins. Accordingly, determination of the concentration of any given chaotropic agent which would be required to cause a structural change in the structural conformation of a native protein at ambient pressure would be straightforward.

In view of the above argument, Dr. Robinson's declaration submitted under 37 C.F.R. § 1.132, and the evidence submitted therewith, Applicants respectfully request that the Examiner withdraw the rejection of claims 3-5, 8, 10 and 11 under 35 U.S.C. § 112, ¶ 2 as being indefinite.

IX. Rejection of Claims 1, 3-5, 7-8, and 10-11 as Anticipated By Gorovits *et al.*

In section 20 of the office action (p. 28), the Examiner rejects claims 1, 3-5, 7-8 and 10-11 under 35 U.S.C. § 102(b) as being anticipated by Gorovits *et al.* (*Biochemistry* 37(17):6132-6135 (1998)). For the reasons noted below, Applicants traverse this rejection.

The claims currently pending in the above referenced application are all directed to a method of recovering native protein from protein aggregates. One step in each of the claimed methods calls for subjecting a sample of protein aggregates to elevated hydrostatic pressure, whereby a portion of protein dissociates from the protein aggregates. Upon return to ambient pressure, native protein is recovered from at least a portion of the protein which dissociated from the aggregate during the elevated hydrostatic pressure step.

In contrast to the Examiner's contentions, Gorovits *et al.* fails to describe subjecting protein aggregates under elevated hydrostatic pressure whereby protein dissociates from the aggregate and is recovered as native protein upon return to ambient pressure. Gorovits *et al.* describes five different experiments.

The first experiment, the procedure of which is described at p. 6133, col. 1, 4th paragraph of the "Experimental Procedure," investigated protein aggregation under pressure. As per the procedure outlined by Gorovits *et al.*, native rhodanese was diluted into a solution having a final urea concentration of 3.9 M and final rhodanese concentration of 0.3 mg/ml. Samples were then pressurized after various times of incubation at pressures varying between 0.001 kbar (ambient pressure) and 2 kbar. The results are shown in Figure 1 on page 6133 and discussed in the first three paragraphs of the "Results and Discussion" section (pp. 6133-6134). Gorovits *et al.* indicate in the second sentence of the "Results and Discussion" that "[a]t 3.9 M urea, the protein is partially unfolded to form a molten globule-like state." In addition, previous work by the co-

author of Gorovits *et al.* (i.e., Paul Horowitz) further confirms that at a concentration of 3.9 M urea, a structural change in the conformation of rhodanese occurs which results in loss of enzymatic activity. See **Exhibit 39**, p. 2501, Figure 1 and accompanying text. Therefore, it follows that native rhodanese could not form in the samples used by Gorovits *et al.* for their first experiment in view of the concentration of urea present in the sample. Because the claims in the present application call for recovery of native protein on return to ambient pressure, the procedure described in the first experiment in Gorovits *et al.* does not anticipate the current claims.

A description of the second experiment of Gorovits *et al.* can be found in the paragraph spanning columns 1-2 on page 6134. As stated in that paragraph:

> The minimum pressure that can prevent protein aggregation was sought.... Here, protein was diluted into *3.9 M urea* and pressurized up to 2 kbar.

(Emphasis added). It follows that the current claims are not anticipated by the second experiment described in Gorovits *et al.* in view of the urea concentration present in the sample used for the second experiment.

A description of the third experiment of Gorovits *et al.* can be found in the first full paragraph of column 2 on page 6134. As stated there:

> A very steep transition was observed in an experiment designed to investigate the equilibrium between low molecular weight forms of rhodanese and protein aggregates at 3.9 M urea .... In this experiment, a rhodanese solution containing *3.9 M urea* was pressurized up to 2 kbar.

(Emphasis added). It follows that the current claims are not anticipated by the third experiment described in Gorovits *et al.* in view of the urea concentration present in the sample used for the third experiment.

A description of the fourth experiment of Gorovits *et al.* can be found in the second full paragraph of column 2 on page 6134. Figure 3 shows the results of the experiment. The caption of Figure 3 states that "[a] protein sample was *prepared as in Figure 1*. Pressure was increased up to 2 kbar ...." (Emphasis added). Since Figure 1 reports the results of the first experiment of Gorovits *et al.*, it follows that the sample used for the fourth experiment contained a concentration of 3.9 M urea. Accordingly, it further follows that the current claims are not anticipated by the fourth experiment described in Gorovits *et al.* in view of the urea concentration present in the sample used for the fourth experiment.

A description of the fifth experiment of Gorovits *et al.* can be found in the last two paragraphs of column 1 on page 6133. According to the procedure, rhodanese was unfolded in 8 M urea for a time sufficient to ensure denaturation and then diluted to 3.6 μg/ml. As further outlined in the last paragraph of column 1 on page 6133: "[t]o investigate rhodanese refolding under high hydrostatic pressure, samples were pressurized up to 2 kbar 5 min after dilution." There is no experimental data in Gorovits *et al.* for the fifth experiment indicating that protein aggregates meeting the functionally defined limitations of the protein aggregates within the scope of the current claims (discussed previously) were present in the sample subjected to hydrostatic pressure. Accordingly, it follows that the current claims are not anticipated by the fifth experiment described in Gorovits *et al.*

X. Rejection of Claims 1-12 as Anticipated By USPN 6,489,450.

In section 21 of the office action (pp. 29-30), the Examiner rejects claims 1-12 under 35 U.S.C. § 102(e) as being anticipated by USPN 6,489,450 (Randolph *et al.*) alone or as evidenced by Paladini *et al.* (*Biochemistry* 20(9):2587-2593 (1981)). For the reasons noted below, Applicants traverse this rejection.

Applicants submit herewith a Declaration Under 37 C.F.R. § 1.131 demonstrating that applicants reduced the claimed invention to practice prior to the earliest available filing date claimed in USPN 6,489,450. In view of their prior invention, applicants respectfully request that the Examiner withdraw the rejection of claims 1-12 under 35 U.S.C. § 102(e) as being anticipated by USPN 6,489,450.

Applicant believes no fee is due with this response. However, if a fee is due, please charge our Deposit Account No. 03-2775, under Order No. 00131-00207-USU from which the undersigned is authorized to draw.

Dated: October 30, 2006

Respectfully submitted,

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